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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 5/11/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head
Section I, Tox. Branch (7509C) Roger Gardner 5/26/94

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Muscular Weakness in the Rat

ACCESSION NO./MRID NO.: 430133-01

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Chlordiazepoxide

STUDY NUMBER: XR2286

REPORT NUMBER: CTL/P/3688

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Assessment of Muscular Weakness in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Chlordiazepoxide hydrochloride (98%) was tested as a positive control in hindlimb and forelimb grip tests in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 5, 10 or 20 mg/kg of the test material in corn oil at a volume of 1 ml/100g bodyweight. The grip strength tests for muscle weakness was conducted one hour after dosing in 3 replicate trials.

The test chemical reduced hindlimb grip strength in both sexes at all dose levels (74 - 83% of controls). In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only (71 - 75% of controls). A comparison of the replicate trials indicated that the data were reproducible.

**No NOEL was established for reduction in hindlimb grip strength and the NOEL for reduction in forelimb grip strength was 10 mg/kg. The LEL for reduction in forelimb grip strength was 20 mg/kg.**

This study is acceptable as a positive control study for the laboratory in which it was conducted. As a general comment, the animals were examined in the fore- and hindlimb grip strength tests at one hour after dosing and not at any time afterwards. For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the $LD_{50}$. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Chlordiazepoxide hydrochloride

Description: Solid
Batch #(s), Other #(s): CTL Ref. No. YO5672/003
Purity: > 98% w/w
Source: Sigma Chemical Company
Vehicle (if applicable): Corn oil

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 216-264g (males); 152-164g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of corn oil.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.



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Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

| Test Group | Dose Administered mg/kg | Main Study male | female |
|---|---|---|---|
| Contr. | 0 | 10 | 10 |
| 1 | 5 | 10 | 10 |
| 2 | 10 | 10 | 10 |
| 3 | 20 | 10 | 10 |

d. Measurement of Forelimb and Hindlimb Grip Strength: One hour following dosing, each rat was tested for muscle relaxation by measuring fore- and hindlimb grip strength. The report stated that the following was used as a procedure: "the apparatus consisted of two strain gauges, one with a triangular ring attached and the second with a T-bar attached, with a perspex channel between. A measurement of grip strength was made by placing tha animal into the channel with its forepaws inside the triangular grasping ring of the forelimb meter. The animal was grasped by the tail and steadily pulled from away from the ring. When the grip was broken the animal was continued to be pulled along the channel so that its hindlimbs grasped the T-bar. The trial was completed when the grip of the hindlimbs was broken." Replicate trials were conducted.

No other measurements were conducted.



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e. Statistical Analyses: Forelimb and hindlimb grip strength were analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Forelimb and Hindlimb Grip Strength

The test chemical reduced hindlimb grip strength in both sexes at all dose levels. In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only. A comparison of the replicate trials indicated that the data were reproducible. The following tables, taken directly from the report summarize the results.

Intergroup Comparison of Grip Strength Data - Post Dosing Males

| | Dose Level of Chlordiazepoxide (mg/kg) | | | |
|---|---|---|---|---|
| Grip Strength | 0 | 5 | 10 | 20 |
| Forelimb, trial 1 | 825 | 827 | 822 | 582** |
| Forelimb, trial 2 | 811 | 743 | 766 | 633** |
| Forelimb, trial 3 | 814 | 724 | 725 | 628** |
| Mean Forelimb | 817 | 765 | 771 | 614** |
| Hindlimb, trial 1 | 652 | 632 | 577 | 501** |
| Hindlimb, trial 2 | 720 | 606* | 557** | 536** |
| Hindlimb, trial 3 | 699 | 597* | 554** | 531** |
| Mean Hindlimb | 690 | 612* | 562** | 523** |

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)



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Intergroup Comparison of Grip Strength Data - Post Dosing Females

| Grip Strength | Dose Level of Chlordiazepoxide (mg/kg) 0 | 5 | 10 | 20 |
|---|---|---|---|---|
| Forelimb, trial 1 | 720 | 765 | 817 | 705 |
| Forelimb, trial 2 | 731 | 735 | 757 | 758 |
| Forelimb, trial 3 | 702 | 710 | 711 | 779 |
| Mean Forelimb | 717 | 736 | 761 | 747 |
| Hindlimb, trial 1 | 641 | 587 | 524** | 517** |
| Hindlimb, trial 2 | 612 | 503* | 476** | 525 |
| Hindlimb, trial 3 | 585 | 543 | 483** | 490* |
| Mean Hindlimb | 612 | 544* | 494** | 511** |

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the hindlimb and forelimb grip strength tests are valid tests for assessment of muscular weakness, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that muscular weakness in the rat can be measured using the hindlimb and forelimb grip tests. The report stated that "the validity of grip strength measurement for the assessment of muscular weakness in the rat has been demonstrated using the known muscle relaxant chlordiazepoxide hydrochloride."



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